

## ACTA BOTANICA CROATICA XXVIII - 1969

THE EFFECT OF AMITROLE ON THE FINE  
STRUCTURE OF ROOT PROPLASTIDS

Mit deutscher und kroatischer Zusammenfassung  
Sa sadržajem na njemačkom i hrvatskom jeziku

MERCEDES WRISCHER and BRANKA VRHOVEC

(Institute »Ruđer Bošković« and Biological Institute of the University, Zagreb)

Received 31 March 1969.

Amitrole (3-amino-1,2,4-triazole) is known as a herbicide which strongly affects developing chloroplasts. The consequence of this process is the formation of chlorotic leaf tissue (Kirk 1967). The effect of amitrole on other types of plastids is not yet well known. Therefore in the present work changes in the fine structure of root proplastids after the treatment with amitrole have been studied.

Two days old seedlings of maize (*Zea mays* L.) and mustard (*Sinapis alba* L.) were taken for the experiments. The concentrations of amitrole used were  $10^{-4}$ ,  $10^{-3}$  and  $10^{-2}$  M. The seedlings were treated with the solution for either 1 or 15 hours. Treated as well as untreated root tips (meristems and zones of cell elongation) were fixed 1 and 3 days after the end of the treatment in 3% glutaraldehyde, postfixed in 1%  $\text{OsO}_4$  and embedded in araldite. Ultrathin sections, made with a Reichert ultramicrotome (type OmU2), were stained with lead citrate (Reynolds 1963) and photographed with a Siemens Elmiskope I.

After an application of either high concentrations ( $10^{-2}$  M, 1 hour), or a prolonged treatment with lower concentrations ( $10^{-4}$  M, 15 hours) treated roots show a delayed growth in length and width, as well as poorly developed root hairs.

Electron microscopic investigations of ultrathin sections have revealed, that only in differentiating cells the proplastids are changed, those of meristematic cells remaining in their ultrastructure always normal. The

proplastids of the rhizodermal cells are sometimes also unchanged. The damaged proplastids are very big, apparently swollen, often of amoeboidal shape and with a very empty stroma (Fig. 2 and 4). Thylakoids and vesicles are also scarce. Starch grains could sometimes still be detected (Fig. 4).

All proplastids of a cell are usually changed to the same degree, but sometimes normal and damaged proplastids occur in the same cell. The percentage of cells with damaged proplastids varies with the concentration of amitrole and the duration of the treatment. The mode of the change is however always the same.

The changes of the proplastids seem to be irreversible. In some cells swollen proplastids exist still 3 days after the end of the treatment. Such proplastids probably disintegrate their outer membrane being after some time ruptured.

According to our experiments other cell organelles seem not to be affected by amitrole treatment. No visible changes could be detected in the fine structure of mitochondria, nuclei, endoplasmic reticulum and Golgi apparatus. The cytoplasm contains always a high number of ribosomes. However an increased number of osmiophilic globules has been observed in the cytoplasm of some cells.

It seems certain — according to our observations — that only the proplastids of the differentiating cells can be damaged by amitrole. Proplastids of the meristematic cells have never been affected, even when examined 3 days after the end of the treatment. These facts seem to be in good agreement with the results obtained on leaves: amitrole affects only chloroplasts in the phase of differentiation (Bartels 1964, Weier and Imam 1965, and Wrischer unpublished). It is known that the protein content of light grown tissue is reduced by amitrole treatment (Bartels 1964). The inhibition of protein synthesis could then prevent the formation of structural elements of the chloroplasts. Ultrastructural changes in the proplastids of the root tissue seem to be caused by similar processes.

## SUMMARY

After a treatment of maize and mustard seedlings with amitrole ( $10^{-4}$  to  $10^{-2}$  M, 1 and 15 hours) in differentiating cells of root tips changes in the fine structure of proplastids have been observed. Such proplastids are much enlarged, apparently swollen and with a very empty stroma. Changes in other organelles have never been observed. The proplastids of the meristematic cells have also never shown any structural changes.

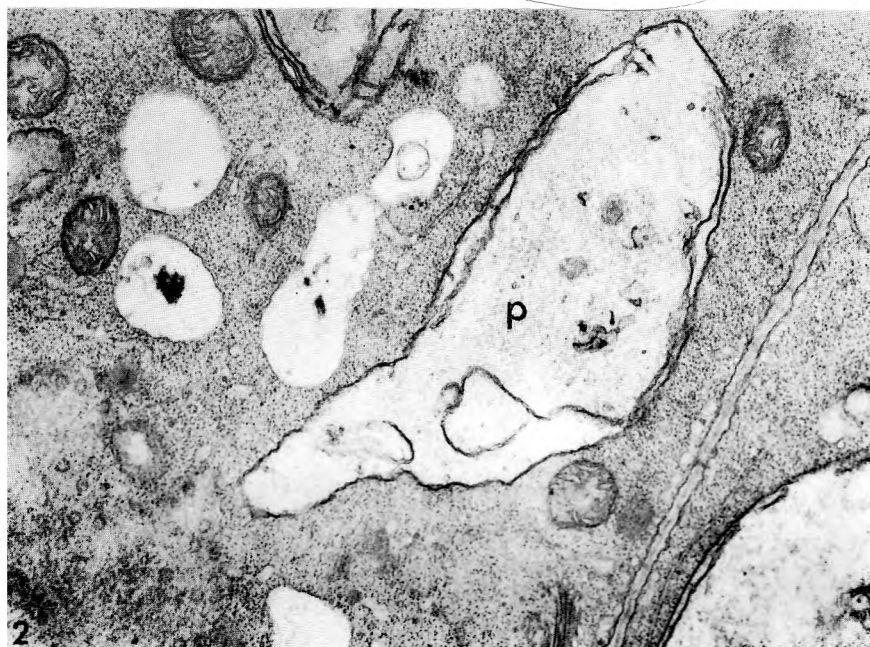
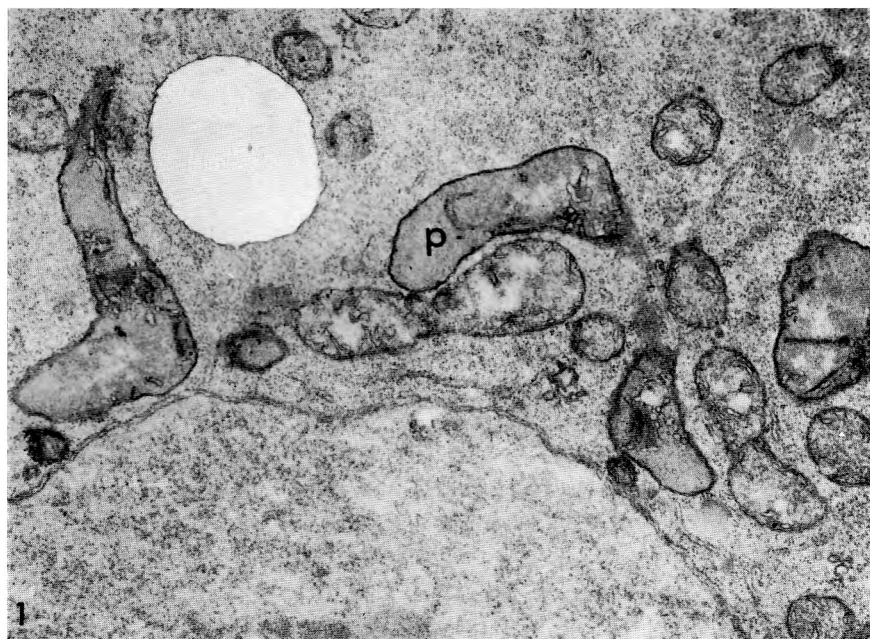
---

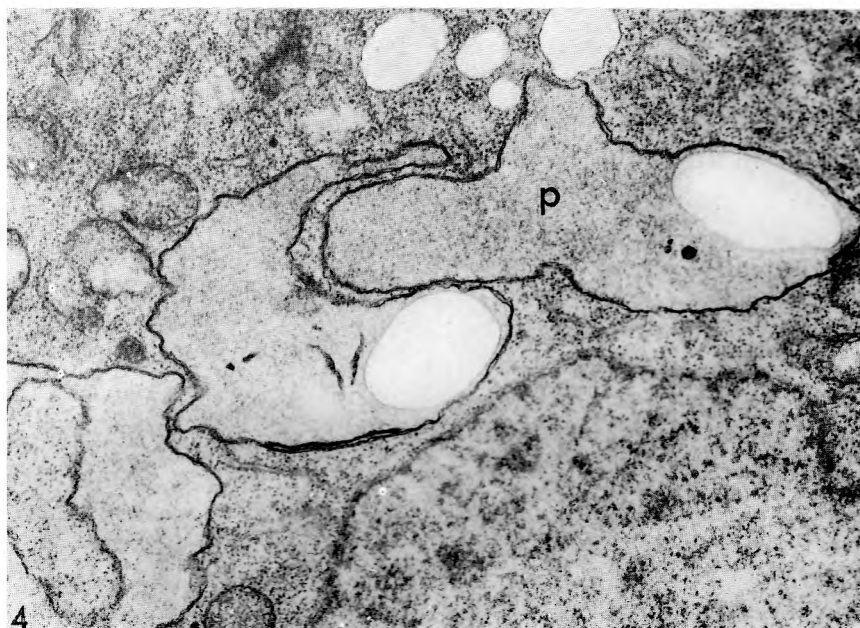
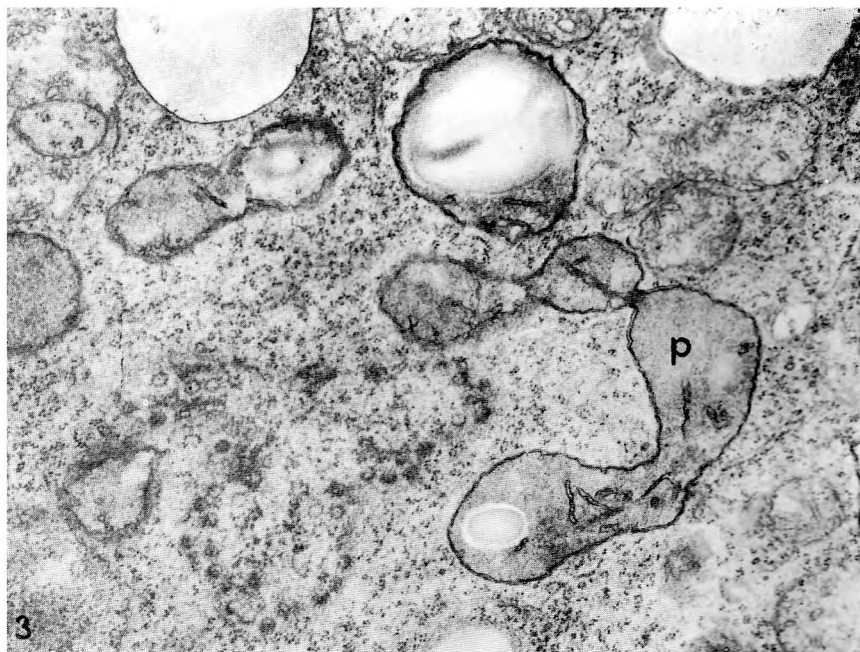
Fig. 1

Part of a differentiating cell from an untreated mustard root tip (control). Proplastids (p) are normal. 16 000 : 1.

Fig. 2

Part of a differentiating cell from a mustard root tip treated 1 hour with a  $10^{-2}$  M solution of amitrole and fixed 1 day after the end of the experiment. Proplastids (p) are big, with an empty stroma and few thylakoids. Other cell organelles are normal. 16 000 : 1.





## References

- Bartels, P. G.*, 1964: The effect of 3-amino-1,2,4-triazole on the chloroplast development and the chemical constituents of wheat seedlings. *Dissert. Abstr.* 25, 2186—2187.
- Kirk, J. T. O. and R. A. E. Tilney-Bassett*, 1967: *The Plastids*. W. H. Freeman and Comp., London and San Francisco.
- Reynolds, E. S.*, 1963: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17, 208—212.
- Weier T. E. and A. A. Imam*, 1965: Some cytological effects of amitrole. *Amer. J. Bot.* 52, 631.



Fig. 3

Part of a differentiating cell from an untreated maize root tip (control).  
Proplastids (p) are normal. 20 000 : 1.

Fig. 4

Part of a differentiating cell from a maize root tip treated 15 hours with a  $10^{-3}$  M solution of amitrole and fixed 1 day after the end of the treatment. Three big, amoeboidal proplastids (p) with an empty stroma and starch grains. Other cell organelles are normal. 20 000 : 1.

## ZUSAMMENFASSUNG

### WIRKUNG VON AMITROL AUF DEN FEINBAU DER WURZEL-PROPLASTIDEN

*Mercedes Wrischer und Branka Vrhovec*

(Institut »Ruder Bošković« und Biologisches Institut der Universität, Zagreb)

Der Einfluss von Amitrol auf die Feinstruktur der Proplastiden in den Zellen der Wurzelspitze von Mais- und Senfkeimlingen wurde elektronenmikroskopisch untersucht. Amitrol scheint nur die Feinstruktur der Proplastiden der sich in Differenzierung befindenden Wurzelzellen zu beeinflussen. Die veränderten Wurzel-Proplastiden sind stark vergrößert, aufgeschwollen, oft amoeboid, mit leerem Stroma und mit spärlichen Thylakoiden oder Bläschen. Der Proplastiden-Feinbau meristematischer Zellen bleibt dagegen immer normal. Ebenso zeigen in allen Zellen andere Organellen nach Behandlung mit Amitrol in der Feinstruktur keine Veränderungen.

## SADRŽAJ

### DJELOVANJE AMITROLA NA ULTRASTRUKTURU PROPLASTIDA KORIJENA

*Mercedes Wrischer i Branka Vrhovec*

(Institut »Ruder Bošković« i Institut za biologiju Sveučilišta u Zagrebu)

Elektronskim mikroskopom ispitivano je djelovanje amitrola na ultrastrukturne promjene proplastida u korijenu kukuruza i gorušice. Istraživanja su pokazala da amitrol uzrokuje upadljive ultrastrukturne promjene samo na proplastidima stanica koje se nalaze u fazi diferencijacije. Promijenjeni proplastidi redovito su vrlo povećani, nabubreni, često ameboidni, s rijetkom stromom i malobrojnim tilakoidima ili vezikulima. U meristemskim stanicama amitrol nema nikakav utjecaj na finu građu proplastida. Ostali organeli u svim stanicama tretiranih biljaka zadrže također posve normalnu ultrastrukturu.